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(54) Title: BETA-SECRETASE ISOLATED FROM HUMAN 293 CELLS

(57) Abstract

Compositions comprising a protease isolated from human 293 cells or polypeptide fragments having about 85 % sequence homology with said protease are disclosed. The native protease is designated β-secretase and cleaves β-amyloid precursor protein on the amino-terminal side of the β-amyloid peptide. The β-secretase has an apparent MW from about 260-300 kD, when glycosylated and binds wheat germ agglutinin. A screening assay for identification of β-secretase inhibitors shows that said β-secretase is not inhibited by common inhibitors of serine, cysteine, aspartyl and metalloproteases.

BETA-SECRETASE ISOLATED FROM HUMAN 293 CELLS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the cleavage of β-amyloid precursor protein to produce β-amyloid peptide. More particularly, the present invention relates to isolated and purified compositions containing an enzyme responsible for such cleavage (β-secretase) and assays for identifying inhibitors of β-secretase.

Alzheimer's disease is characterized by the presence of numerous amyloid plaques and neurofibrillary tangles (highly insoluble protein aggregates) present in the brains of Alzheimer's disease patients, particularly in those regions involved with memory and cognition. β-amyloid peptide is a major constituent of amyloid plaque which is produced by cleavage of β-amyloid precursor protein. It is presently believed that a normal (non-pathogenic) processing of the β-amyloid precursor protein occurs via cleavage by a putative "α-secretase" which cleaves between amino acids 16 and 17 of the β-amyloid peptide region within the protein. It is further believed that pathogenic processing occurs in part via a putative "β-secretase" which cleaves at the amino-terminus of the β-amyloid peptide region within the precursor protein. Heretofore, however, the existence of β-secretase has not been confirmed.

The identification, isolation, and characterization of novel biological molecules having unique activities is generally useful. For example, novel enzymes can be used to catalyze reactions of a type associated with their class. In particular, novel proteases can be used to cleave proteins for a variety of purposes, and the availability of new proteases provides unique capabilities. In addition to such uses associated with enzymes in general, the identification,

terminus of β-amyloid peptide (βAP) within APP, referred to hereinafter as "β-secretase activity." The compositions of the present invention will generally have a β-secretase activity which is at least five-fold greater than that of a solubilized but unenriched membrane fraction from human 293 cells, preferably being at least ten-fold greater than that of the membrane fraction, and more preferably being at least 100-fold greater than that of the membrane fraction. The β-secretase enzyme is characterized by (1) an apparent molecular weight when glyosylated in the range from 260 kD to 300 kD when measured by gel exclusion chromatography in 9.2% hydrogenated Triton X-100, (2) a gel pattern as shown in Figs. 1 or 2 (discussed in more detail in the Experimental section hereinafter), (3) a net negative charge at pH 5 and a net negative charge at pH 7.5, and (4) binding to wheat germ agglutinin. In the native and isolated forms, β-secretase is glycosylated. Methods for deglycosylation are described in the Experimental section hereinafter. The above apparent molecular weight is determined prior to any deglycosylation.

The compositions of the present invention are generally useful as proteolytic chemical and specifically useful in assays for determining whether a test substance will inhibit proteolytic cleavage of APP resulting from the novel β-secretase. The method comprises exposing a polypeptide comprising the β-secretase site of APP (located at the amino-terminus of the βAP region within APP) to an at least partially purified β-secretase in the presence of the test substance under conditions such that the β-secretase would be expected to cleave the polypeptide into an amino-terminal fragment and a carboxy-terminal fragment in the absence of test substance which inhibits such cleavage. Test substances which inhibit such cleavage are thus identified as having β-secretase inhibition activity. Such test methods preferably employ the β-secretase compositions described above. Usually, generation of the amino-terminal fragment and/or the carboxy-terminal amino acids of APP, and may comprise a fusion polypeptide including an amino-terminal portion having a binding epitope. Use of such a fusion

Fig. 8 is an anionic exchange column elution profile obtained for deglycosylated β-secretase enzyme.

Fig. 9 is a Western blot of the deglycosylated β-secretase enzyme probed with 238B antisera.

Fig. 10 is an elution profile of the β-secretase enzyme from a cationic exchange column.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides a novel protease which cleaves the β-amyloid precursor protein (APP) at the amino-terminus of the β-amyloid peptide (βAP) therein. It is believed that this protease is the putative β-secretase responsible for the pathogenic processing of APP to produce βAP in βAP-related conditions, such as Alzheimer's disease, Down's syndrome, HCHWA-D, and the like. Thus, the novel protease of the present invention will be referred to hereinafter as "β-secretase." The β-secretase of the present invention will be useful as a protease in *in vitro* and *in vivo* systems where proteases may generally find use. For example, β-secretase may be used to cleave or attempt to cleave proteins in order to characterize, process, modify, or otherwise react with the protein as a substrate. Thus, β-secretase will have general utility as a proteolytic chemical reagent in a wide variety of chemical reactions and systems. In addition, the β-secretase of the present invention will have a specific utility in the performance of screening assays to identify β-secretase inhibitors, *i.e.*, test compounds which are able to inhibit the proteolytic cleavage of APP in the presence of β-secretase. Such assays will be described in detail below.

As used herein, "β-amyloid precursor protein" (APP) refers to a polypeptide that is encoded by a gene of the same name localized in humans on the long arm of chromosome 21 and that includes a βAP region (defined below) within its carboxyl third. APP is a glycosylated, single-membrane-spanning protein expressed in a wide variety of cells in many mammalian tissues. Examples of specific isotypes of APP which are currently known to exist in humans are the 695-amino acid

form of APP. Proteolytic activity appears to be at its peak at a pH from 5 to 5.5, with very low activity at pH 7.5 and above. β-secretase is resistant to many known protease inhibitors (see Table 1 in the Experimental section below). β-secretase appears to recognize only those polypeptide substrates which have retained a substantial number of residues upstream and downstream from the cleavage site (from either the wild-type, Swedish, or other mutated form) of APP, with an oligopeptide analog including 17 residues upstream and 16 residues downstream from the cleavage site (with a total of 33 amino acids) being resistant to cleavage by β-secretase.

The β-secretase of the present invention will be provided in an isolated and purified form. By "isolated and purified," it is meant that the β-secretase has been either (1) isolated and at least partially purified from a natural source, such as human brain tissue or human 293 cells (as described in detail in the Experimental section below) or (2) is produced recombinantly and synthetically. At present, as neither the amino acid sequence nor the nucleic acid sequence of the β-secretase gene have been determined, it is preferred that β-secretase be obtained from cellular sources using known protein purification techniques. Contaminating proteins may be removed from the β-secretase co-positions by specific techniques, including serial lectin chromatography on agarose-bound succinylated-wheat germ agglutinin (SWGA) and agarose-bound lentil lectin (LCA). These lectins, although partly binding β-secretase activity, preferentially bind other contaminating proteins in the purified fractions, and thus allow increased enrichment of the β-secretase activity.

The β-secretase will be isolated and purified to an extent sufficient to increase the β-secretase activity in the resulting composition to a useful level. In particular, the β-secretase preparations of the present invention will have sufficient activity to cleave APP and APP-containing polypeptides as described in the Experimental section below. Preferably, the β-secretase compositions of the present invention will have an activity which is at least 10-fold greater than that of a solubilized but unenriched membrane

be useful for labelling reagents, purification targets, affinity ligands targeting, or the like.

The β-secretase polypeptides of the present invention may be used to prepare polyclonal and/or monoclonal antibodies using conventional techniques with the β-secretase as an immunogen. The intact β-secretase molecule, or fragments thereof, optionally coupled to a carrier molecule, may be injected into small vertebrates, with monoclonal antibodies being produced by well-known methods. See, for example, Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, New York, and Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2nd ed.) Academic Press, New York. Antibodies produced from β-secretase will be useful for performing conventional immunoassays to detect β-secretase in biological and other specimens.

The β-secretase compositions described above will be particularly useful for performing *in vitro* assays for detecting β-secretase inhibitors, where at least partially purified β-secretase is combined with the polypeptide substrate comprising the β-secretase cleavage site of APP in the presence of the test substrate. Conditions are maintained such that the β-secretase would cleave the polypeptide substrate into an amino-terminal fragment and a carboxy-terminal fragment in the absence of a substance which inhibits such cleavage. Cleavage of the polypeptide substrate in the presence of the test compound is compared with that in the absence of the test compound, and those test substances which provide significant inhibition of the cleavage activity (usually at least about 25% inhibition, more usually at least about 50% inhibition, preferably at least about 75% inhibition, and often at least about 90% inhibition or higher) are considered to be β-secretase inhibitors. Such β-secretase inhibitors may then be subjected to further *in vitro* and/or *in vivo* testing to determine if they inhibit the production of βAP in cellular and animal models. Suitable *in vivo* and *in vitro* tests are described in copending application Serial Nos.

polypeptide is detected using conventional labelling systems, such as horseradish peroxidase or other detectable enzyme labels, which are bound to the antibody directly (covalently), or indirectly through intermediate linking substances, such as biotin and avidin.

The following examples are offered by way of illustration, not by way of limitation.

EXPERIMENTAL

Purification and Characterization of β-Secretase

Frozen tissue (293 cell paste or human brain) was cut into pieces and combined with five volumes of homogenization buffer (20 mM Hepes, pH 7.5, 0.25 M sucrose, 2 mM EDTA). The suspension was homogenized using a blender and centrifuged at 1000 x g (10 min, 4°C) to produce a post-nuclear supernatant which was saved on ice. The pellets were resuspended in fresh homogenizing buffer at the original volume, and the centrifugation step was repeated. The second supernatant was combined with the first one, and the supernatant pool ("PNS") was centrifuged at 16,000 x g for 30 min at 4°C. The supernatants were discarded and the pellets, labelled "P2," were either used immediately for enzyme purification or frozen at -40°C for later use.

The pellets were suspended in extraction buffer (20 mM MES, pH 6.0, 0.5% Triton X-100, 150 mM NaCl, 2 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml E64, 1 μg/ml pepstatin, 0.2 mM PMSF) at the original volume. After vortex-mixing, the extraction was completed by agitating the tubes at 4°C for a period of one hour. The mixtures were centrifuged as above at 16,000 x g, and the supernatants were pooled. The pH of the extract was adjusted to 7.5 by adding ~1% (v/v) of 1 M Tris base (not neutralized).

The neutralized extract was loaded onto a wheat germ agglutinin-agarose (WGA-agarose) column pre-equilibrated with 10 column volumes of 20 mM Tris, pH 7.5, 0.5% Triton X-100, 150 mM NaCl, 2 mM EDTA, at 4°C. One milliliter of the agarose resin was used for every 4 g of original tissue used. The WGA-column was washed with 10 column volumes of the

Superdex 200 (26/60) gel exclusion chromatography column, which was eluted with phosphate buffered saline, pH 7.4, 0.2% hydrogenated Triton X-100, 2 mM EDTA, at 1 ml/min, collecting 3 min/fraction. Fractions containing β-secretase activity were identified using the MBP-C125 cleavage assay. The apparent molecular weight of the β-secretase activity eluting from the Superdex column was estimated from the peak elution volume (relative to that of standard proteins) to be 280,000 ± 9800 (average of two runs for 293 cells, and two runs for human brain).

Results from a large-scale preparation of the enzyme from human brain tissue is shown in Table 1 below.

Table 1

Step	Activity ng/ml/h	Protein µg/ml	Sp. Act.[1] ng/ml/h/µg protein	Fold Purfn.
Solubilized membrane extr.	2700	350	7.7	1
HiQ Elution pool	80000	210	380.9	49.5
Con A Flow-Thru	80000	100	800	103.8
Superdex peak fraction	57000	< 5	> 11400	> 1480.5

[1] Specific activity of the purified β-secretase was measured as follows. MBP C125-SW (described below) was combined at approximately 0.7 µg/ml in 100 mM sodium acetate, pH 5.5, with 0.3% Triton X-100. The amount of product generated was measured by the β-secretase assay, also described below. Specific activity was then calculated as:

$$Sp.Act. = \frac{(Product\ conc.\ ng/ml)(Dilution\ factor)(Incubation\ vol.\ \mu l)}{(Enzyme\ sol.\ vol.\ \mu l)(Incubation\ time\ h.)(Enzyme\ conc.\ \mu g/ml)}$$

The Sp. Act. is thus expressed as ng of protein produced per µg of β-secretase per hour.

In order to analyze for protein, a portion (360 µl) of each fraction was concentrated by acetone precipitation,

Table 2

Lectin	β-Secretase Binding
jequirity bean (APA)	+
jack bean (con A)	+
scotch broom (CSA)	+
jimson weed (DSA)	+
coral tree (ECA)	-
grifornia simplicifolia I	-
grifornia simplicifolia II	-
Jacalin (AIA)	+
lentil (LCA)	+
horseshoe crab (LPA)	-
tomato (LEL)	+
maackia (MAA)	+
peanut (PNA)	+
pokeweed (POA)	-
castor bean (RCA1)	-
potato (STL)	-
wheat germ - succinylated (SWGA)	+
China gourd (TKA)	+
stinging nettle (UDA)	+
gorse (UEAI)	-
gorse (UEAII)	-
hairy vetch (VVL)	-
wheat germ (WGA)	+++

Only a single lectin (WGA) bound β-secretase activity quantitatively, out of the many tested. Partial binding of the activity (25-40%) to a number of other lectins probably indicates heterogeneous glycosylation.

Table 3

Inhibitor	Max Conc	IC50
SERINE PROTEASES		
aminoethylbenzene-sulfonyl fluoride	0.8 mM	NI
chymostatin	0.2 mM	NI
3,4-dichloroisocoumarin	0.5 mM	< 25% inh.
diisopropylfluoro-phosphate	2 mM	NI
elastatinal	0.2 mM	NI
phenylmethylsulfonyl-fluoride	1.0 mM	NI
CYSTEINE PROTEASES		
E-64	0.14 mM	NI
N-ethylmaleimide	10 mM	NI
iodoacetamide	10 mM	NI
METALLOPROTEASES		
EDTA	2 Mm	NI
phosphoramidon	10 mM	NI
o-phenanthroline		7 mM
m-phenanthroline		7 mM
ASPARTYL PROTEASES		
pepstatin	25 μM	NI
diazoacetylnorleucyl-methyl ester		> 5 mM
DIVALENT METAL IONS		
Cu		2 mM
Zn		3 mM
Hg		< 10% inh
Ca		NI
Mg		NI

The eluate from the DEAE-Sepharose beads (~250:1) was then treated with 1/20 (v/v) of recombinant PNGase F (Glyko, 2.5 U/ml) for 4-5 d at 37°C, in the presence of 3 mM β-mercaptoethanol. This treatment removed Asn-linked carbohydrate chains from glycoproteins.

Anion-exchange chromatography was then performed as follows. The deglycosylated enzyme sample was diluted 1:10 in MiniQ Buffer A, and loaded on to a MiniQ PC3.213 (Pharmacia) anion-exchange column equilibrated with this buffer. The column was washed with 10 volumes of 30 mM NaCl in MiniQ Buffer A, followed by elution with a linear gradient to 500 mM NaCl in Buffer A over 20 min, at a flow rate of 0.4 ml/min. Fractions (0.4 ml) were analyzed for both β-secretase activity utilizing the MBP-C125Sw cleavage assay (Fig 8), as well as immunoreactivity for the previously detected polypeptide using antisera 238B analyzed by Western blotting after Tris-Tricine PAGE on 10-20% acrylamide Novex gels (Fig 9). Fractions (7-10) corresponding to the peak of enzymatic activity contained partially resolved forms of the polypeptides recognized by the 238B antisera, ranging in apparent MW from between approx 48 kDa to approx 148 kDa.

Cation-exchange chromatography was used to further purify the enzymatic activity. The peak activity fractions from the MiniQ elution were diluted 1:5 in SP Buffer A (20 mM NaOAc, pH 4.75, 2 mM EDTA, 0.2% R-TX100) in order to alter the pH as well as lower the ionic strength prior to cation exchange chromatography. The diluted enzyme pool was mixed with 50:1 of a 50% slurry of Pharmacia SP-Sepharose® beads. Following overnight mixing at 4°C, the beads were collected by low speed centrifugation, and the supernatant ("SP Flow-Through") was saved for further analysis. The SP-Sepharose beads were eluted stepwise with 100 mM NaCl increments in SP Buffer A, up to 600 mM NaCl. These fractions (E1-E6, ~0.45 ml each), along with the SP Flow-Through, and an aliquot of the SP Load, were analyzed for both β-secretase activity as well as immunoreactivity for the previously identified polypeptide. The results are shown in Fig 10. All of the immunoreactive bands bind to the SP-Sepharose, since they are quantitatively

purified substrates were stored frozen at -40°C in 3 M guaninide-HCl and 1% Triton X-100, @ ~0.7 mg/ml.

Microtiter 96-well plates were coated with purified anti-MBP antibody (@ 5-10 μg/ml), followed by blocking with human serum albumin. β-secretase solution (1-10 μl) is mixed with substrate (0.5 μl) in a final volume of 50 μl, with a final buffer composition of 20 mM sodium acetate, pH 5.5, 0.03% Triton X-100, in individual wells of 96-well microtiter plates, and incubated at 37°C for 2 h. Samples were then diluted 5-fold with Specimen Diluent (0.2 g/l sodium phosphate monobasic, 2.15 g/l sodium phosphate dibasic, 0.5 g/l sodium azide, 8.5 g/l sodium chloride, 0.05% Triton X-405, 6 g/l BSA), further diluted 5-10 fold into Specimen Diluent on anti-MBP coated plates, and incubated for 1 h. Biotinylated SW192 antibodies were used as the reporter. SW192 polyclonal antibodies were biotinylated using NHS-biotin (Pierce), following the manufacturer's instruction. Usually, the biotinylated antibodies were used at about 240 ng/ml, the exact concentration varying with the lot of antibodies used. Following incubation of the plates with the reporter, the ELISA was developed using streptavidin-labeled alkaline phosphatase (Boeringer-Mannheim) and 4-methyl-umbelliferyl phosphate as fluorescent substrate. Plates were read in a Cytofluor 2350 Fluorescent Measurement System. Recombinantly generated MBP-26SW (product analog) was used as a standard to generate a standard curve (Fig. 7), which allowed the conversion of fluorescent units into amount of product generated.

This assay protocol was used to screen for inhibitor structures, using "libraries" of compounds assembled onto 96-well microtiter plates. Compounds were added, in a final concentration of 20 μg/ml in 2% DMSO, in the assay format described above, and the extent of product generated compared with control (2% DMSO only) β-secretase incubations, to calculate "% inhibition." "Hits" were defined as compounds which result in >35% inhibition of enzyme activity at test concentration. Using this system, 70 "hits" were identified out of the first 6336 compounds tested, a "hit" rate of ~1.1%.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Athena Neurosciences, Inc.

(ii) TITLE OF INVENTION: Beta-Secretase

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(D) STATE: California
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(F) ZIP: 94111-3834

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(B) FILING DATE: herewith
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Heslin, James M.
(B) REGISTRATION NUMBER: 29,541
(C) REFERENCE/DOCKET NUMBER: 015270-002230PC

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 415-326-2422

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Val Lys Met Asp Ala
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

TTTGAGCAGA TGCAGAACTA G 1521

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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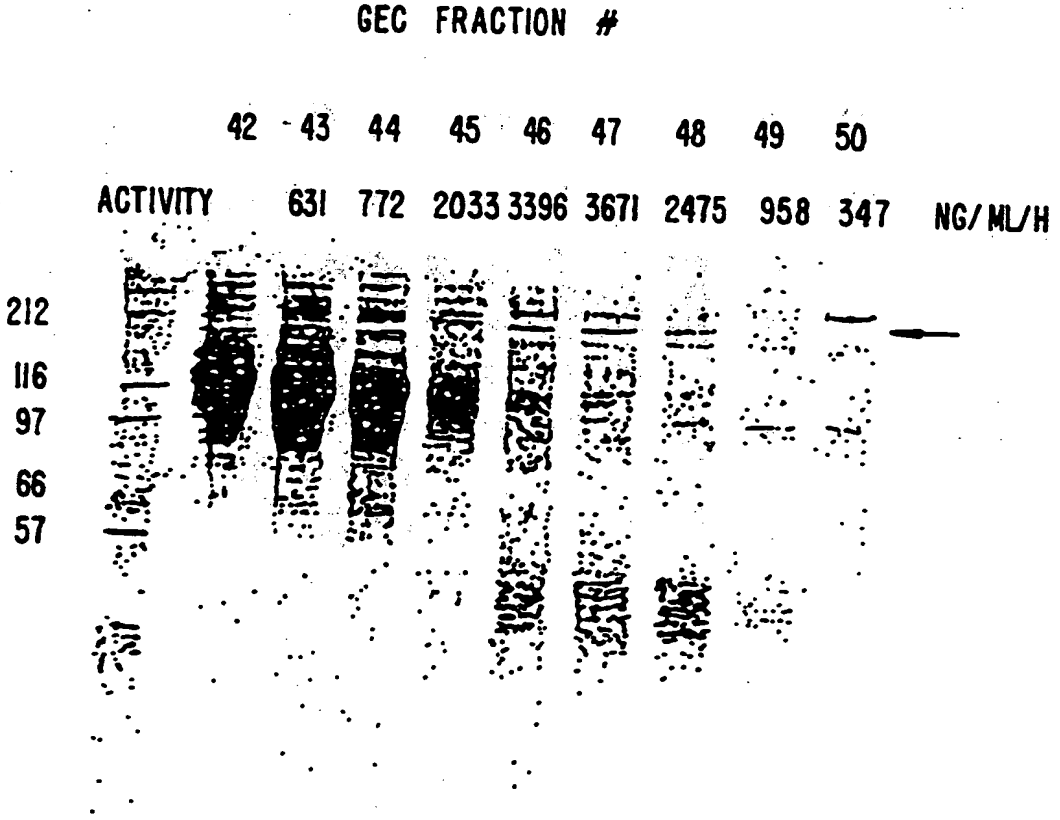
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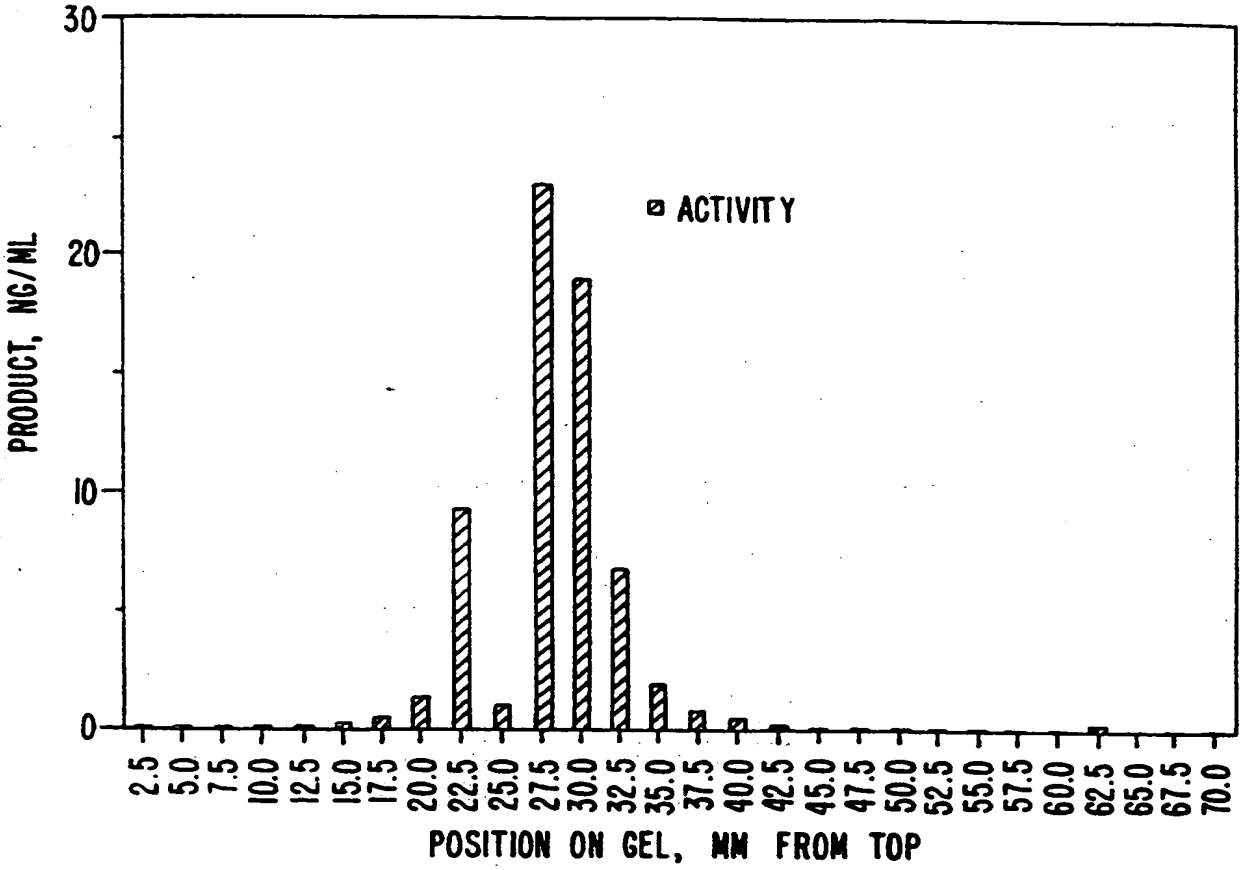
FIG. 1.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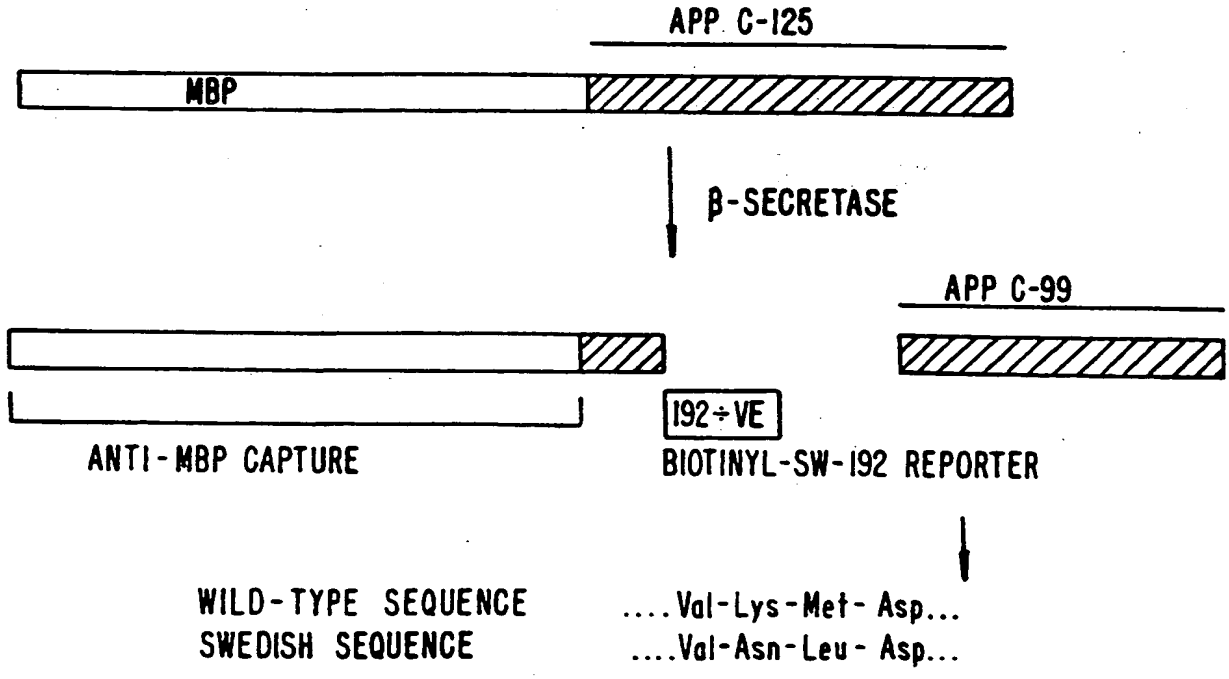
POSITION ON GEL, MM FROM TOP

FIG. 3.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MBP
β-SECRETASE
APP C-99
192÷VE
ANTI-MBP CAPTURE
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SWEDISH SEQUENCE ....Val-Asn-Leu-Asp...

FIG. 4.

```
   1 ATGAAAACTGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAGTCGGTAAG
   1 MetLysThrGluGluGlyLysLeuValIleTrpIleAsnGlyAspLysGlyTyrAsnGlyLeuAlaGluValGlyLys

  79 AAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCACAGGTTGCG
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 157 GCAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCT
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 235 GAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGCTG
  79 GluIleThrProAspLysAlaPheGlnAspLysLeuTyrProPheThrTrpAspAlaValArgTyrAsnGlyLysLeu

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 391 GAAGAGATCCCGGCGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTAC
 131 GluGluIleProAlaLeuAspLysGluLeuLysAlaLysGlyLysSerAlaLeuMetPheAsnLeuGlnGluProTyr

 469 TTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTACGACATTAAAGACGTG
 157 PheThrTrpProLeuIleAlaAlaAspGlyGlyTyrAlaPheLysTyrGluAsnGlyLysTyrAspIleLysAspVal

 547 GGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACCTGATTAAAAACAAACACATGAATGCAGAC
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 625 ACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCC
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 703 AACATCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCGTT
 235 AsnIleAspThrSerLysValAsnTyrGlyValThrValLeuProThrPheLysGlyGlnProSerLysProPheVal
```

FIG. 6-1.

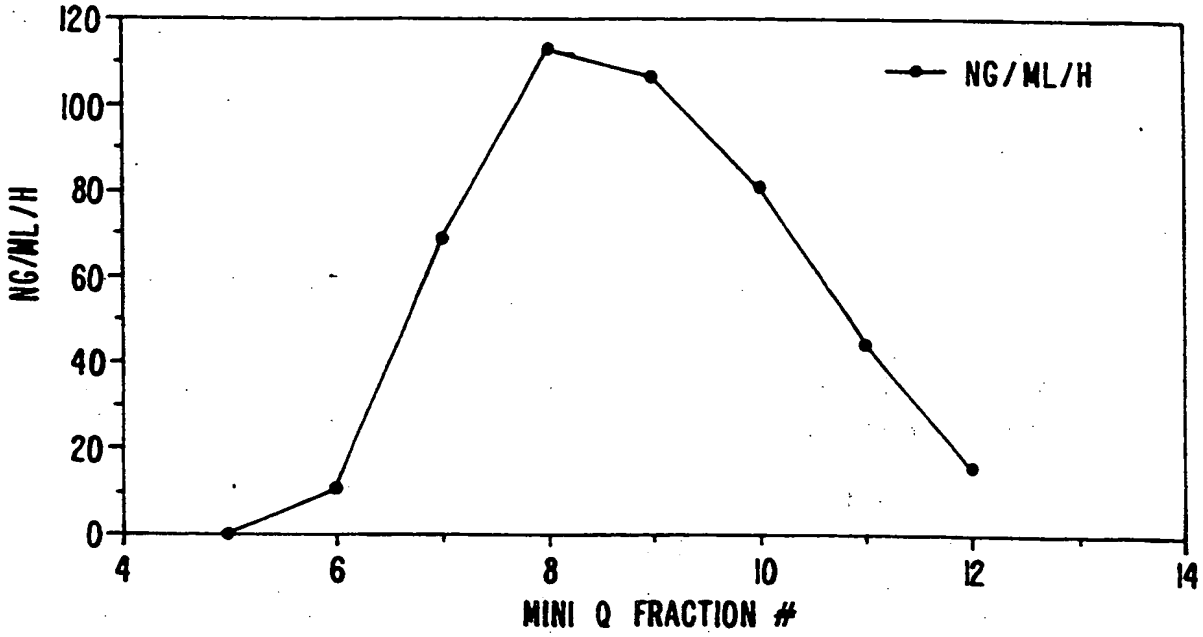
120
100
80
60
40
20
0
NG/ML/H
NG/ML/H
4
6
8
10
12
14
MINI Q FRACTION #

FIG. 8.

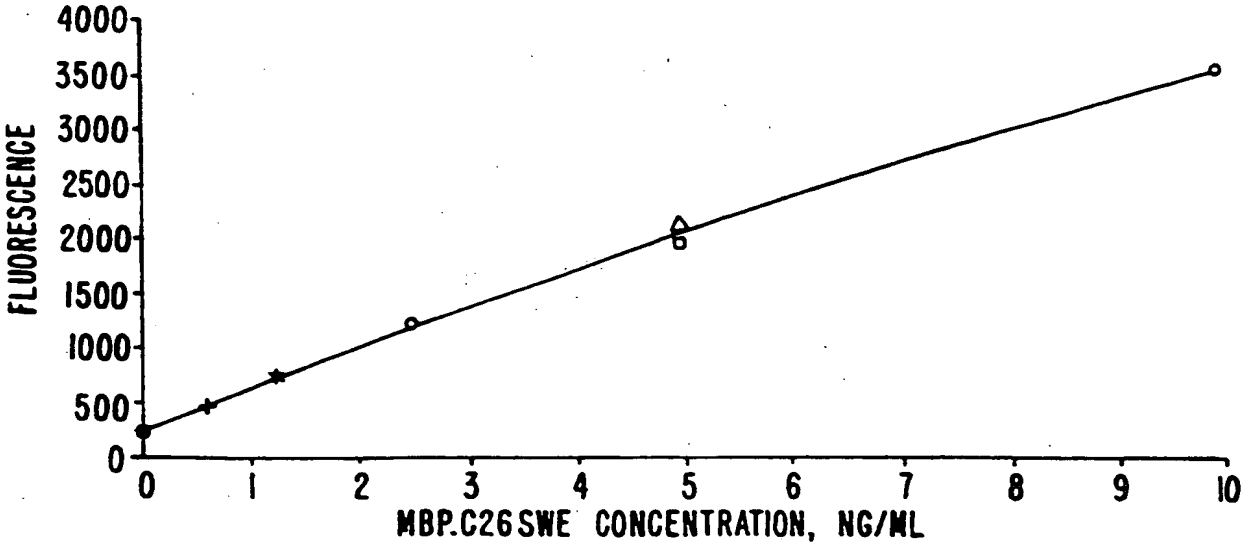
4000
3500
3000
2500
2000
1500
1000
500
0
FLUORESCENCE
0
1
2
3
4
5
6
7
8
9
10
MBP.C26SWE CONCENTRATION, NG/ML

FIG. 7.

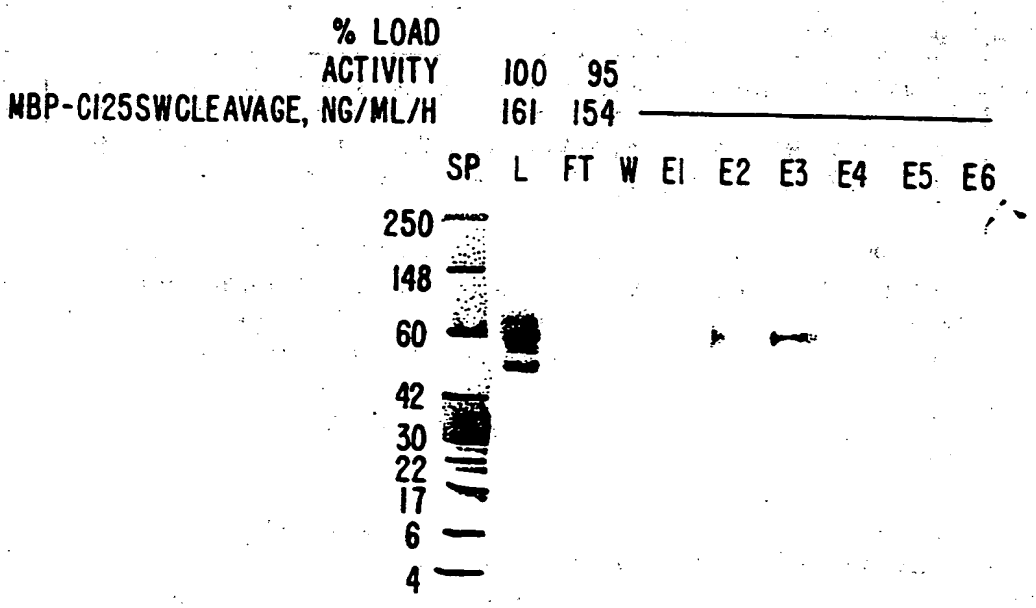
% LOAD
ACTIVITY 100 95
MBP-C125SWCLEAVAGE, NG/ML/H 161 154
SP L FT W E1 E2 E3 E4 E5 E6
250
148
60
42
30
22
17
6
4

FIG. 10.

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US96/19549

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NELSON et al. Identification of a chymotrypsin-like mast cell protease in rat brain capable of generating the N-terminus of the Alzheimer amyloid β-protein. Journal of Neurochem. 1993, Vol. 61, No. 2, pages 567-577, especially pages 571-572.	1
A	SAMBAMURTI et al. Evidence for intracellular cleavage of the Alzheimer's amyloid precursor in PC12 cells. Journal of Neurosci. Res. 1992, Vol. 33, pages 319-329, entire document.	1-4